

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 December 2001 (06.12.2001)

PCT

(10) International Publication Number
WO 01/92513 A1

- (51) International Patent Classification⁷: C12N 15/11 (74) Agent: BALDWIN SHELSTON WATERS; 60 Margaret Street, Sydney, NSW 2000 (AU).
- (21) International Application Number: PCT/AU01/00627
- (22) International Filing Date: 29 May 2001 (29.05.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
PQ7830 30 May 2000 (30.05.2000) AU (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- PQ9246 7 August 2000 (07.08.2000) AU (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
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Published:

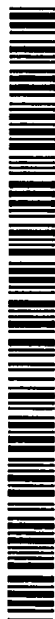
— with international search report

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(54) Title: METHODS FOR MEDIATING GENE SUPPRESSION BY USING FACTORS THAT ENHANCE RNAi

(57) Abstract: The present invention is concerned with methods for enhancing gene suppression in cells and in particular it is concerned with improved methods for enhancing RNAi-mediated gene silencing by manipulation of factors associated with RNAi. The present invention is also concerned with methods for identifying factors which down-regulate as well as those which up-regulate RNAi. It is also concerned with genetic constructs useful for enhancing or modulating gene silencing and cells harbouring such constructs.

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Methods for mediating gene suppression by using factors that enhance RNAi**TECHNICAL FIELD**

The present invention is concerned with methods for enhancing gene suppression in cells and in particular it is concerned with improved methods for enhancing RNAi-mediated gene silencing by manipulation of factors associated with RNAi. The present invention is also concerned with methods for identifying factors which down-regulate as well as those which up-regulate RNAi. It is also concerned with genetic constructs useful for enhancing or modulating gene silencing and cells harbouring such constructs.

BACKGROUND OF THE INVENTION

The use of double-stranded RNA (dsRNA) to specifically interfere with gene expression has received considerable attention because of its demonstrated potency in a range of organisms, including some which have so far been genetically intractable. Termed RNA interference (RNAi), it has been implicated in viral defense, control of transpositional elements, genetic imprinting and endogenous gene regulation. It has been hypothesised to be the central mechanism in post-transcriptional gene silencing (PTGS), co-suppression, quelling, and antisense RNA-mediated gene suppression. One model that has been proposed is that dsRNA is fragmented into 21-25 nt species by dsRNA-specific nucleases, amplified by RNA-dependent RNA polymerase, and then dissociated and free to attack homologous mRNA by RNA nuclease-mediated degradation. The application of this technique will greatly facilitate the dissection of gene function and the validation of genes involved in disease states.

Recently at least two different strategies have been undertaken to identify the cellular proteins composing a proposed multi-protein complex involved in the recognition of dsRNA and the activation of dsRNA-mediated gene interference. The first involves the use of classical chemical mutagenesis or insertional mutagenesis to isolate mutants completely defective for RNAi and cloning of the relevant genes using complementation. These studies have been carried out in genetically tractable organisms such as plants, worms and fungi. The genetic screens described involve the use of RNAi systems in which the degree of suppression is complete. The mutagenesis produces mutants in which the RNAi effect is completely reversed indicating the loss of a cellular factor (function) required for the RNAi effect. Thus these genetic screens would most

likely miss factors that have subtle effects or rate limiting or rate determining roles in RNAi.

The second strategy for finding key players in RNAi has involved the use of cell free assays. These in vitro reconstitution assays, on the other hand, identify cellular factors that impact on RNAi outside of the cellular context and therefore the cellular role of these factors must always be tested.

However, the major disadvantages of these strategies are that genes will not be identified if they are essential to the organism, nor will they directly identify gene activities which will enhance RNAi when overexpressed.

Thus there is a need for models which can demonstrate a range of RNAi efficacies, with both increasing and decreasing quantitative activities being selectable. This would enable the identification of factors which can enhance or reduce the gene silencing effect.

It is therefore an object of the present invention to overcome or at least ameliorate one or more disadvantages of the prior art, or provide a useful alternative.

SUMMARY OF THE INVENTION

Through the use of a fission yeast model for the study of dsRNA-mediated gene silencing and in the search for factors involved in this process, it was surprisingly found that the natural level of RNAi activity can be enhanced by manipulating factors associated with RNAi activity or efficacy. Thus, it has been found that by increasing the steady-state levels of a target nucleic acid sequence in the presence of the same pool of the corresponding antisense sequence, or a part thereof, the antisense-mediated suppression was not only maintained, but enhanced. This is indicative of an RNAi-like mechanism of gene suppression. It has also been found that overexpression of certain sequences, named herein RNAi enhancing sequences (*res*), (also referred to herein as anti-sense enhancing sequences – *aes*), also had the ability to enhance RNAi.

This ability of RNAi activity to be enhanced in *S. pombe* provides a model system which enables the analysis of RNAi processes and the identification and study of factors which either up-regulate or down-regulate its activity. The system has been further used to identify RNAi enhancing gene sequences which increase PTGS efficacy when their resulting protein activities are augmented in vivo. The model used to exemplify the present invention and the methods described are also applicable to

treatment of disorders in which gene expression requires more efficient modulation or silencing.

According to a first aspect there is provided a method for inhibiting the expression of a target nucleic acid in a cell, which method comprises the steps of

- 5 (i) elevating in the cell the level of an RNAi factor, and
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid
- 10 inhibits expression of the target nucleic acid.

- According to a second aspect there is provided a method of increasing cellular susceptibility to anti-sense-mediated inhibition of target nucleic acid expression, which method comprises elevating the level of an RNAi factor in a cell that expresses said target nucleic acid, with the proviso that the cell is to have prior, concurrently or
- 15 subsequently introduced thereinto a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.

- According to a third aspect there is provided a method for treating a subject
- 20 suffering from a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises the steps of

- (i) elevating the level of an RNAi factor in the subject's cells where the target nucleic acid is expressed, and
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into
- 25 such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid, thereby treating the subject.

- According to a fourth aspect there is provided a method for inhibiting in a
- 30 subject the onset of a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises the steps of
 - (i) elevating the level of an RNAi factor in the subject's cells where the target nucleic acid would be expressed if the subject were suffering from the disorder, and

- (ii) prior, concurrently with or subsequent to performing step (i), introducing into such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid would inhibit expression of the target nucleic acid were such expression to occur, thereby inhibiting in the subject the onset of the disorder.

According to a fifth aspect there is provided a method of determining whether inhibiting the expression of a particular target nucleic acid or the activity of its product may alleviate a disorder, which method comprises the steps of

- (i) elevating the level of an RNAi factor in a cell whose phenotype correlates with that of a cell from a subject having the disorder;
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid; and
- (iii) determining whether the cell's phenotype now correlates with that of a cell from a subject in whom the disorder has been alleviated or the disorder is not evident, thereby determining whether inhibiting the expression of the target nucleic acid or the activity of its product may alleviate the disorder.

In a preferred embodiment the target nucleic acid is an endogenous nucleic acid or a part thereof, but it may also be an exogenous sequence or part thereof.

Preferably the level of the RNAi factor is elevated by introducing into the cell additional copies of, or agents which give rise to, the RNAi factor. It will be understood therefore that up-regulating the expression of an endogenous RNAi factor will also achieve the same result and is contemplated herein as part of the invention.

Preferably the factor is selected from the group consisting of a gene, cDNA, RNA or a protein. More preferred is a factor selected from the group consisting of a transcriptional activator of the antisense nucleic acid, a component of the RNAi machinery, a component of the DNA replication machinery and a component of translational machinery. Even more preferred is an RNAi factor which is an *res* sequence.

Also for preference the factor can be selected from the group consisting of ATP-dependent RNA helicase (*ded1*), transcriptional factor *thi1*, DNA replication protein *sna41*, ribosomal protein L7a, elongation factor EF-Tu and *res1* as herein defined.

Further preferred factors are represented by the *res* sequences which are
5 obtainable from transformed cells designated herein W18, W20, W21, W23, W27, W28, W30, W32 and W47.

Preferably the *res* sequence is represented by any one of Seq ID Nos 1 to 4.

The preferred cell is a eukaryotic cell and even more preferred is a mammalian cell. In certain embodiments of the invention described herein the preferred cell is a
10 *Schizosaccharomyces pombe* cell.

Preferably the antisense nucleic acid corresponds to a part only of the target nucleic acid.

According to a sixth aspect there is provided a pharmaceutical composition for use in performing the method of any one of the previous aspects comprising

- 15 (i) an expressible nucleic acid encoding, or capable of increasing or decreasing the expression of, an RNAi factor;
- (ii) a nucleic acid encoding a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid; and
- 20 (iii) a pharmaceutically acceptable carrier,
- wherein the nucleic acids of (i) and (ii) may be situated on the same or different molecules.

According to a seventh aspect there is provided a pharmaceutical composition for use in performing the method of any one of claims 2 to 17 comprising

- 25 (i) a nucleic acid which is the target nucleic acid or a part thereof, or an expressible nucleic acid encoding a factor capable of elevating the intracellular level of the target nucleic acid;
- (ii) a nucleic acid encoding a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid; and
- 30 (iii) a pharmaceutically acceptable carrier,
- wherein the nucleic acids of (i) and (ii) may be situated on the same or different molecules.

According to an eighth aspect there is provided a cell having increased susceptibility to anti-sense-mediated inhibition of a target nucleic acid expression, which cell (i) expresses a target nucleic acid and (ii) comprises an elevated level of an RNAi factor, with the proviso that the cell is to have introduced therein a molecule
5 which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.

For preference the cell is a eukaryotic cell but more preferred is a mammalian
10 cell. As indicated above, in certain embodiments of the invention described herein the preferred cell is a *Schizosaccharomyces pombe* cell.

According to a ninth aspect there is provided a method for inhibiting the expression of a target nucleic acid in a cell, which method comprises the steps of
(i) augmenting the level of the target nucleic acid or a part thereof in the cell, and
15 (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of said target nucleic acid, under conditions permitting an increase in the degree to which the anti-sense nucleic acid inhibits expression of said target nucleic acid.

20 According to a tenth aspect there is provided a method of increasing cellular susceptibility to anti-sense-mediated inhibition of a target nucleic acid expression, which method comprises augmenting the level of the target nucleic acid or a part thereof in a cell expressing the target nucleic acid, with the proviso that the cell is to have prior, concurrently or subsequently introduced therein a molecule which is, or
25 gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions permitting the increase in the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.

According to an eleventh aspect there is provided a method for treating a subject suffering from a disorder whose alleviation is mediated by inhibiting the expression of a
30 target nucleic acid, which method comprises the steps of
(i) augmenting the level of said target nucleic acid or a part thereof in the subject's cells where the target nucleic acid is expressed, and

CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for inhibiting the expression of a target nucleic acid in a cell, which method comprises the steps of
 - (i) elevating in the cell the level of an RNAi factor, and
 - 5 (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.
- 10 2. A method of increasing cellular susceptibility to anti-sense-mediated inhibition of target nucleic acid expression, which method comprises elevating the level of an RNAi factor in a cell that expresses said target nucleic acid, with the proviso that the cell is to have prior, concurrently or subsequently introduced therein to a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the
15 RNA transcript of the target nucleic acid under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.
3. A method for treating a subject suffering from a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises
20 the steps of
 - (i) elevating the level of an RNAi factor in the subject's cells where the target nucleic acid is expressed, and
 - (ii) prior, concurrently with or subsequent to performing step (i), introducing into
25 such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid, thereby treating the subject.
4. A method for inhibiting in a subject the onset of a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises
30 the steps of
 - (i) elevating the level of an RNAi factor in the subject's cells where the target nucleic acid would be expressed if the subject were suffering from the disorder, and

(ii) prior, concurrently with or subsequent to performing step (i), introducing into such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid would inhibit expression of the target nucleic acid were such expression to occur, thereby inhibiting in the subject the onset of the disorder.

5. A method of determining whether inhibiting the expression of a particular target nucleic acid or the activity of its product may alleviate a disorder, which method comprises the steps of

- 10 (i) elevating the level of an RNAi factor in a cell whose phenotype correlates with that of a cell from a subject having the disorder;
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions
- 15 permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid; and
- (iii) determining whether the cell's phenotype now correlates with that of a cell from a subject in whom the disorder has been alleviated or the disorder is not evident, thereby determining whether inhibiting the expression of the target nucleic acid or the
- 20 activity of its product may alleviate the disorder.

6. A method according to any one of claims 1 to 5, wherein the target nucleic acid is an exogenous nucleic acid or a part thereof.

7. A method according to any one of claims 1 to 6, wherein the level of the RNAi factor is elevated by introducing into the cell additional copies of, or agents which give

25 rise to, the RNAi factor.

8. A method according to any one of claims 1 to 7, wherein the factor is selected from the group consisting of a gene, cDNA, RNA or a protein.

9. A method according to any one of claims 1 to 8, wherein the factor is selected from the group consisting of a transcriptional activator of the antisense nucleic acid, a

30 component of the RNAi machinery, a component of the DNA replication machinery and a component of translational machinery.

10. A method according to any one of claims 1 to 9, wherein the RNAi factor is an *res* sequence.
11. A method according to claim 10, wherein the factor is selected from the group consisting of ATP-dependent RNA helicase (*ded1*), transcriptional factor *thi1*, DNA
5 replication protein *sna41*, ribosomal protein *L7a*, elongation factor EF-Tu and *res1* as herein defined.
12. A method according to claim 11, wherein the *res* sequence is obtainable from transformed cells designated herein W18, W20, W21, W23, W27, W28, W30, W32 and W47.
- 10 13. A method according to claim 11, wherein the *res* sequence is represented by Seq ID Nos 1 to 4.
14. A method according to any one of claims 1 to 13, wherein the cell is a eukaryotic cell.
15. A method according to 14, wherein the eukaryotic cell is a mammalian.
- 15 16. A method according to claim 1 or claim 2, wherein the cell is a *Schizosaccharomyces pombe* cell.
17. A method according to any one of claims 1 to 16, wherein the antisense nucleic acid corresponds to a part of the target nucleic acid.
18. A pharmaceutical composition for use in performing the method of any one of
20 claims 2 to 17 comprising
- (i) an expressible nucleic acid encoding, or capable of increasing or decreasing the expression of, an RNAi factor;
- (ii) a nucleic acid encoding a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic
25 acid; and
- (iii) a pharmaceutically acceptable carrier,
- wherein the nucleic acids of (i) and (ii) may be situated on the same or different molecules.
19. A pharmaceutical composition for use in performing the method of any one of
30 claims 2 to 17 comprising
- (i) an nucleic acid which is the target nucleic acid or a part thereof, or an expressible nucleic acid encoding a factor capable of elevating the intracellular level of the target nucleic acid;

(ii) a nucleic acid encoding a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid; and

(iii) a pharmaceutically acceptable carrier,

5 wherein the nucleic acids of (i) and (ii) may be situated on the same or different molecules.

20. A cell having increased susceptibility to anti-sense-mediated inhibition of a target nucleic acid expression, which cell (i) expresses a target nucleic acid and (ii) comprises an elevated level of an RNAi factor, with the proviso that the cell is to have
10 introduced therein to a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions permitting the RNAi factor to increase the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.

21. A cell according to claim 20, wherein the cell is a eukaryotic cell.

15 22. A cell according to claim 20 or claim 21, wherein the cell is a *Schizosaccharomyces pombe* cell.

23. A method for inhibiting the expression of a target nucleic acid in a cell, which method comprises the steps of

(i) augmenting the level of the target nucleic acid or a part thereof in the cell, and

20 (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of said target nucleic acid, under conditions permitting an increase in the degree to which the anti-sense nucleic acid inhibits expression of said target nucleic acid.

25 24. A method of increasing cellular susceptibility to anti-sense-mediated inhibition of a target nucleic acid expression, which method comprises augmenting the level of the target nucleic acid or a part thereof in a cell expressing the target nucleic acid, with the proviso that the cell is to have prior, concurrently or subsequently introduced therein to a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a
30 portion of the RNA transcript of the target nucleic acid under conditions permitting the increase in the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid.

25. A method for treating a subject suffering from a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises the steps of

- (i) augmenting the level of said target nucleic acid or a part thereof in the subject's
5 cells where the target nucleic acid is expressed, and
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under
10 conditions permitting an increase in the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid, thereby treating the subject.

26. A method for treating a subject suffering from a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises the steps of

- (i) augmenting the level of the target nucleic acid or a part thereof in the subject's
15 cells where the target nucleic acid is expressed, and
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under
20 conditions permitting an increase in the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid, thereby treating the subject.

27. A method for inhibiting in a subject the onset of a disorder whose alleviation is mediated by inhibiting the expression of a target nucleic acid, which method comprises the steps of

- (i) augmenting the level of the target nucleic acid or a part thereof in the subject's
25 cells where the target nucleic acid would be expressed if the subject were suffering from the disorder, and
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into such cells a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid, under
30 conditions permitting an increase in the degree to which the anti-sense nucleic acid would inhibit expression of the target nucleic acid were such expression to occur, thereby inhibiting in the subject the onset of the disorder.

28. A method of determining whether inhibiting the expression of a particular target nucleic acid or the activity of its product may alleviate a disorder, which method comprises the steps of

- (i) augmenting the level of the target nucleic acid in a cell whose phenotype correlates with that of a cell from a subject having the disorder;
- (ii) prior, concurrently with or subsequent to performing step (i), introducing into the cell a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid under conditions permitting an increase in the degree to which the anti-sense nucleic acid inhibits expression of the target nucleic acid; and
- (iii) determining whether the cell's phenotype now correlates with that of a cell from a subject in whom the disorder has been alleviated or the disorder is not evident, thereby determining whether inhibiting the expression of the target nucleic acid or the activity of its product may alleviate the disorder.

29. A method according to any one of claims 23 to 28, wherein the target nucleic acid is an exogenous nucleic acid or a part thereof.

30. A method according to any one of claims 23 to 29, wherein the level of the target nucleic acid is augmented by introducing into the cell additional copies of, or agents which are capable of inducing intracellular over-expression of, the target nucleic acid.

31. A method according to any one of claims 23 to 30, wherein the cell is a eukaryotic cell.

32. A method according to 31, wherein the eukaryotic cell is a mammalian.

33. A method according to claim 23 or claim 24, wherein the cell is a

Schizosaccharomyces pombe cell.

34. A method according to any one of claims 23 to 33, wherein the antisense nucleic acid corresponds to a part of the target nucleic acid.

35. A cell having increased susceptibility to anti-sense-mediated inhibition of a target nucleic acid expression, which cell (i) expresses said target nucleic acid and (ii) comprises an elevated level of said target nucleic acid, with the proviso that the cell is to have introduced therein a molecule which is, or gives rise to, an anti-sense nucleic acid directed toward at least a portion of the RNA transcript of the target nucleic acid

under conditions permitting the RNAi factor to increase the degree to which the antisense nucleic acid inhibits expression of the target nucleic acid.

36. A cell according to claim 35, wherein the cell is a eukaryotic cell.

37. A cell according to claim 35 or claim 36, wherein the cell is a

5 *Schizosaccharomyces pombe* cell.

38. Method of identifying a cellular factor capable of effecting and/or modulating expression of a target nucleic acid in a cell having the target nucleic acid and a nucleic acid which is an antisense of the target nucleic acid or part thereof, which method comprises over-expressing said factor in the cell and wherein the expression of the
10 target nucleic acid is capable of being enhanced or only partially suppressed.

39. A factor identified by the method of claim 38.

40. A factor according to claim 39, wherein the factor is selected from the group consisting of a gene, cDNA, RNA or a protein.

41. A factor according to claim 39 or claim 40, wherein the factor is selected from
15 the group consisting of a transcriptional activator or the antisense nucleic acid, a component of the RNAi machinery, a component of the DNA replication machinery and a component of translational machinery.

42. A factor according to any one of claims 39 to 41, wherein the factor is an *res* sequence.

20 43. A factor according to claim 42, wherein the factor is selected from the group consisting of ATP-dependent RNA helicase (*ded1*), transcriptional factor *thi1*, DNA replication protein *sna41*, ribosomal protein L7a, elongation factor EF-Tu and *res1* as herein defined.

44. An RNAi factor which is an *res* sequence obtainable from transformed cells
25 designated herein W18, W20, W21, W23, W27, W28, W30, W32 and W47.

45. An RNAi factor which is an *res* sequence represented by Seq ID Nos 1 to 4.

46. A *Schizosaccharomyces pombe* cell having a target nucleic acid or a part thereof and a antisense nucleic acid or a part thereof which corresponds to the target nucleic acid or a part thereof, wherein the expression of the target nucleic acid is capable of
30 being enhanced or only partially suppressed.

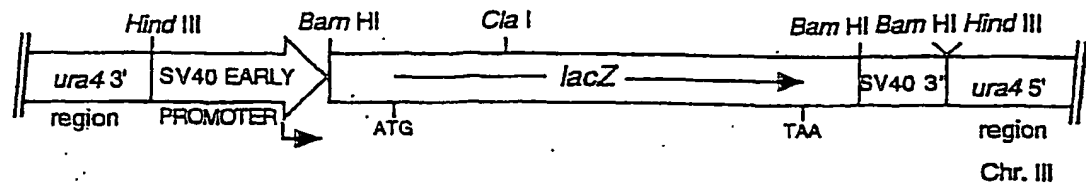
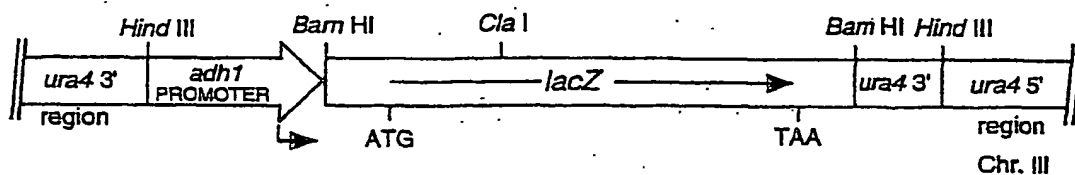
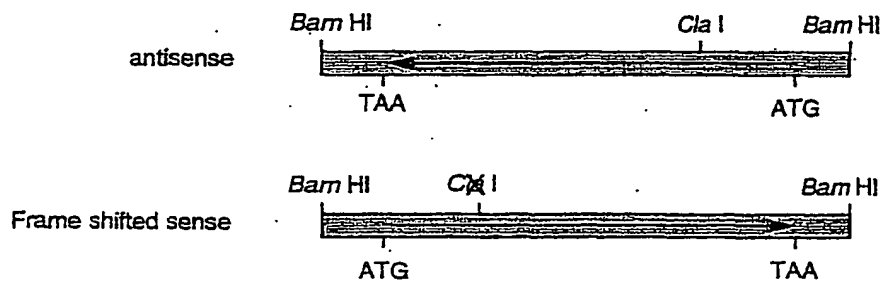
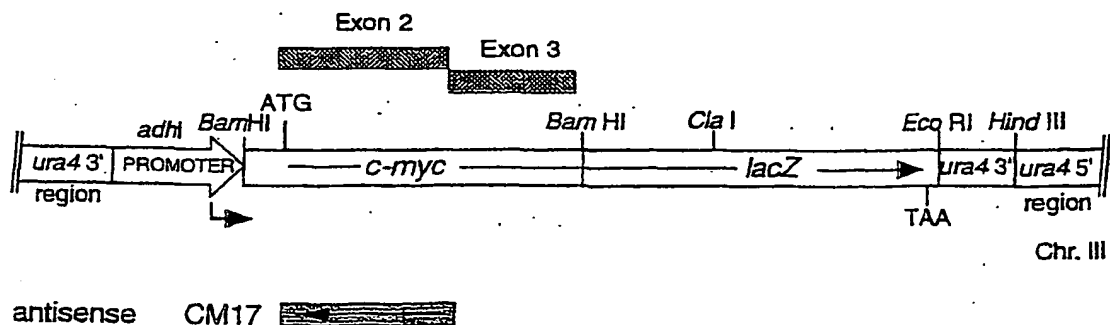
A Target gene: KC4-6 strain**B** Target gene: RB3-2 strain**C** *lacZ* antisense and sense fragments**D** Target gene fusion: AML1 strain and antisense fragment

Figure 1

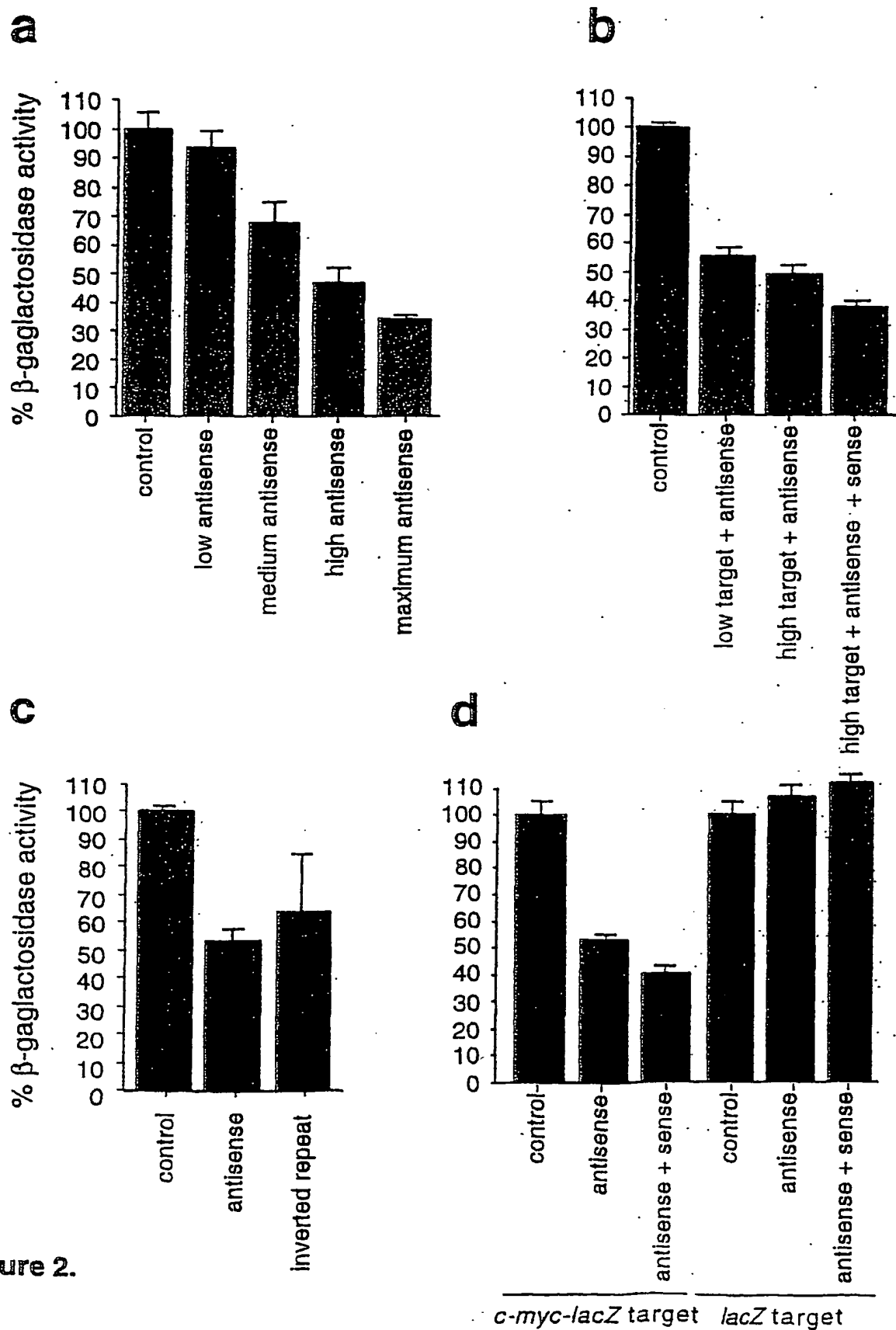


Figure 2.

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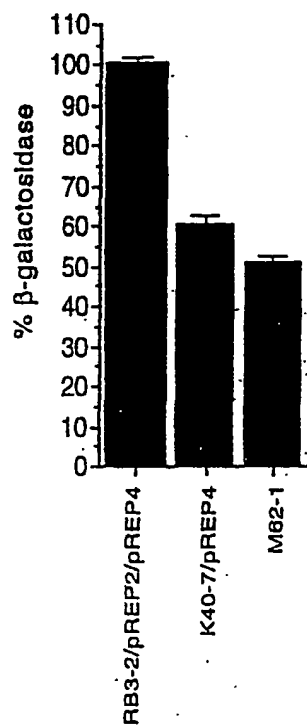


Figure 3

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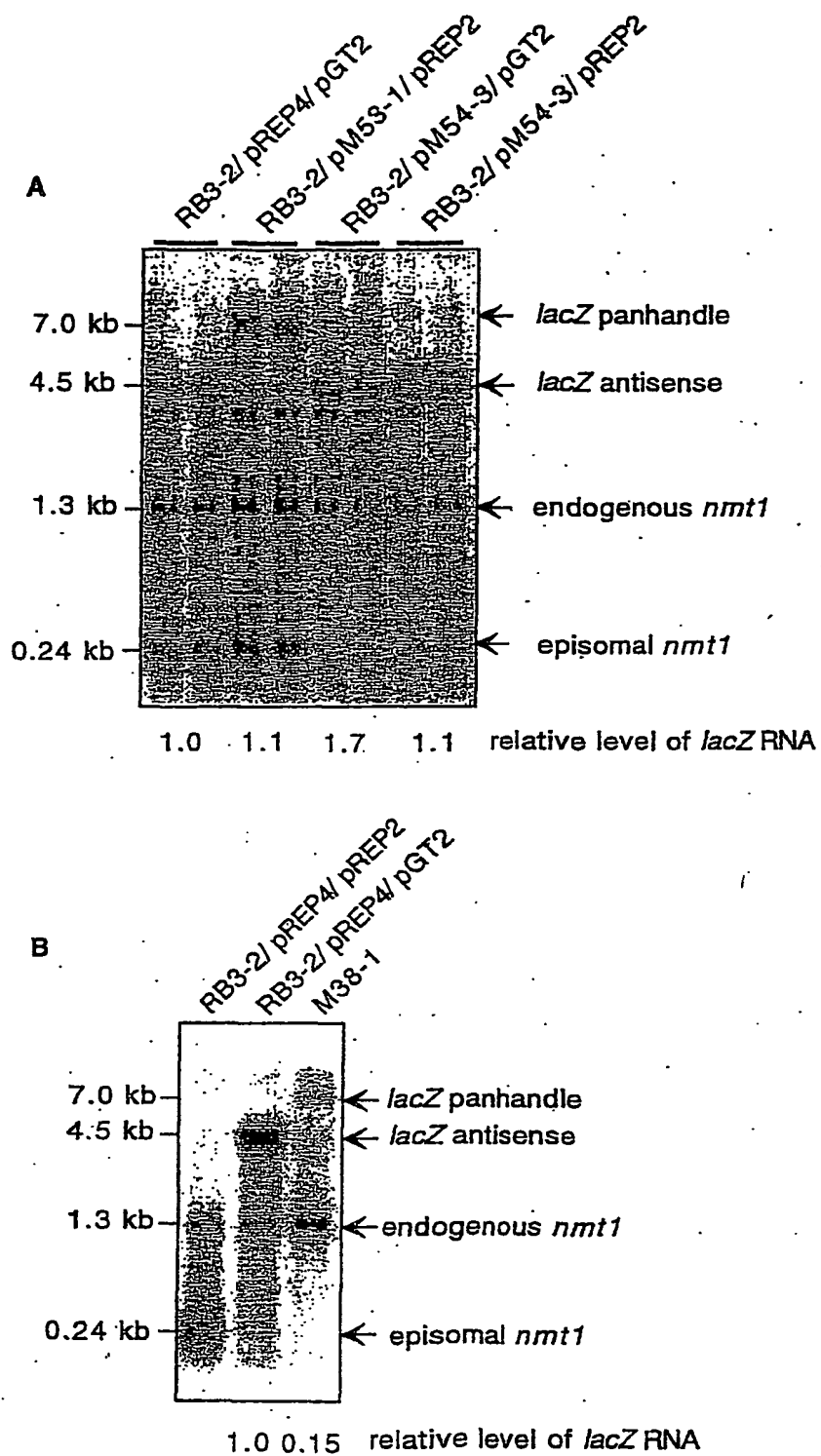


Figure 4

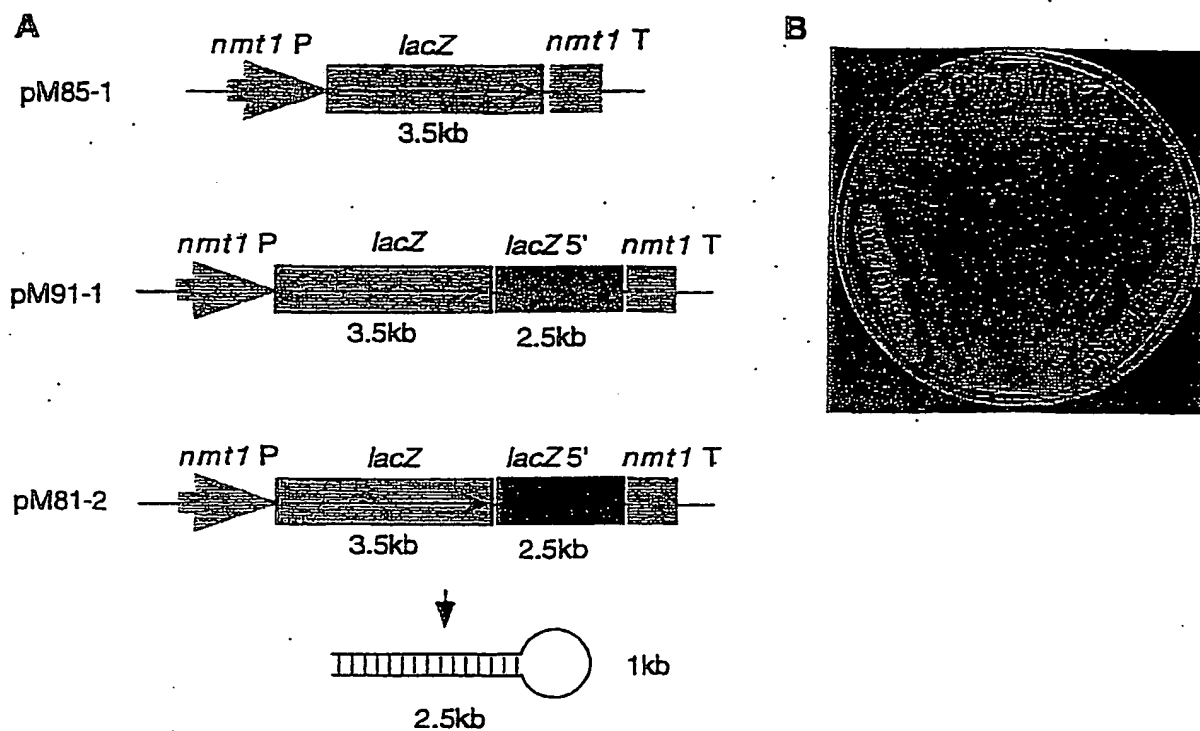


Figure 5

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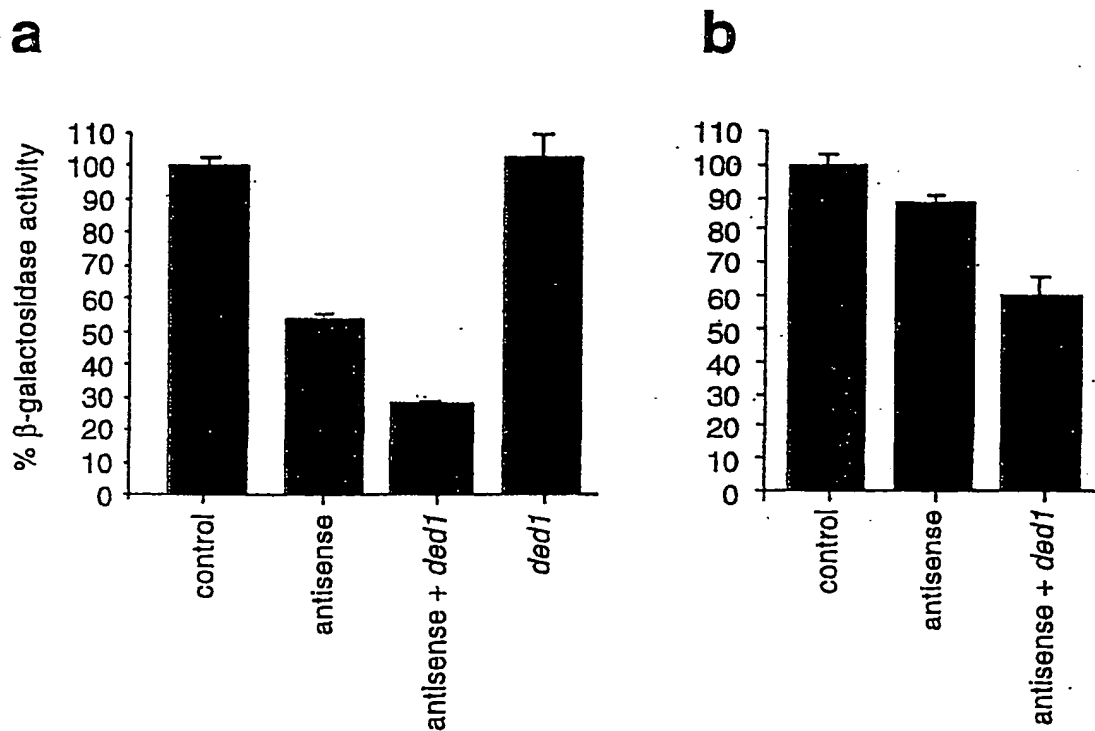


Figure 6.

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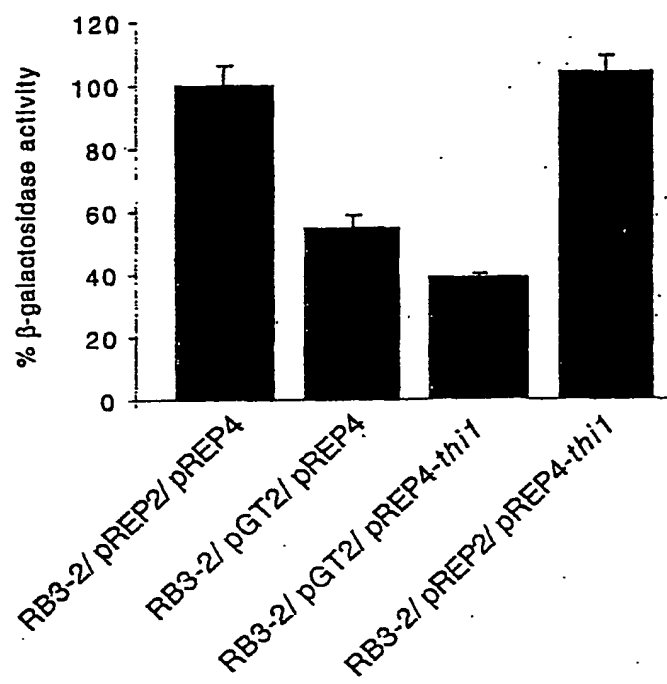


Figure 7

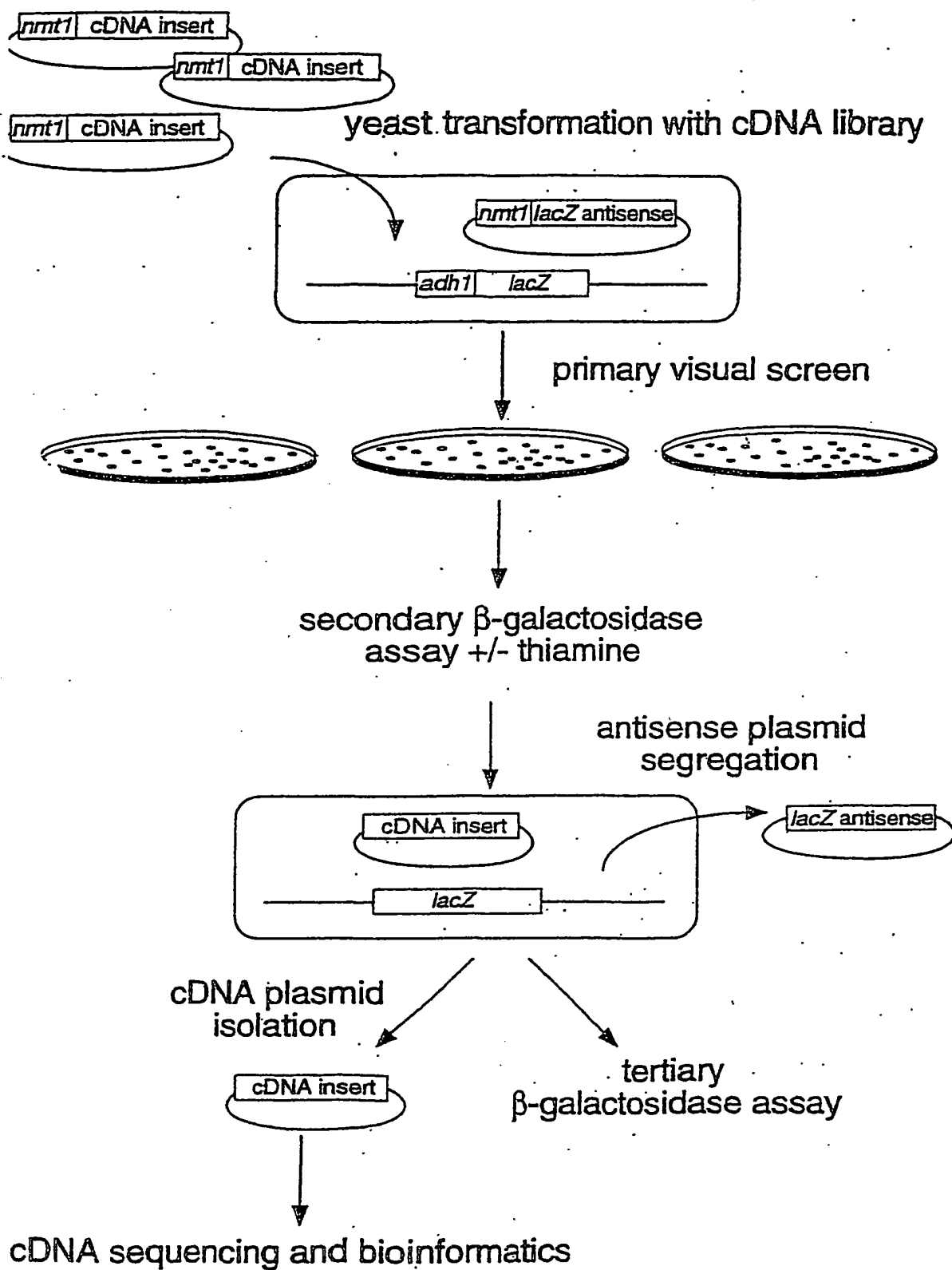
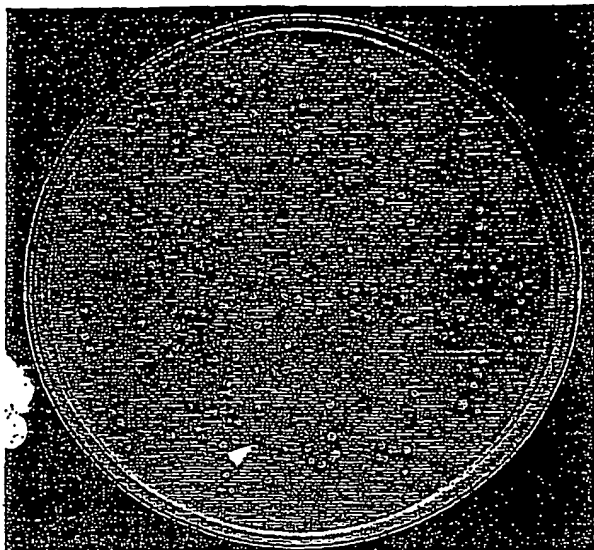


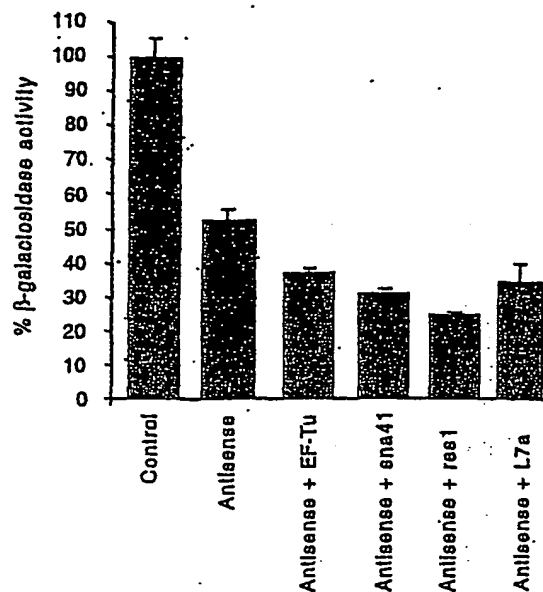
Figure 8.

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a



c



b

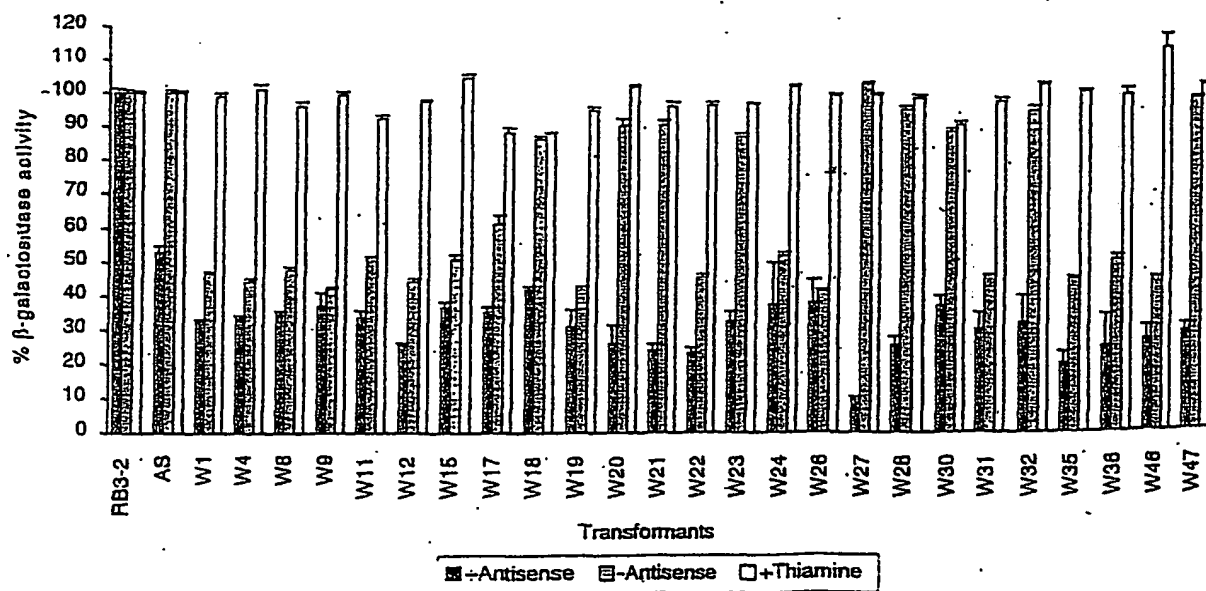


Figure 9.

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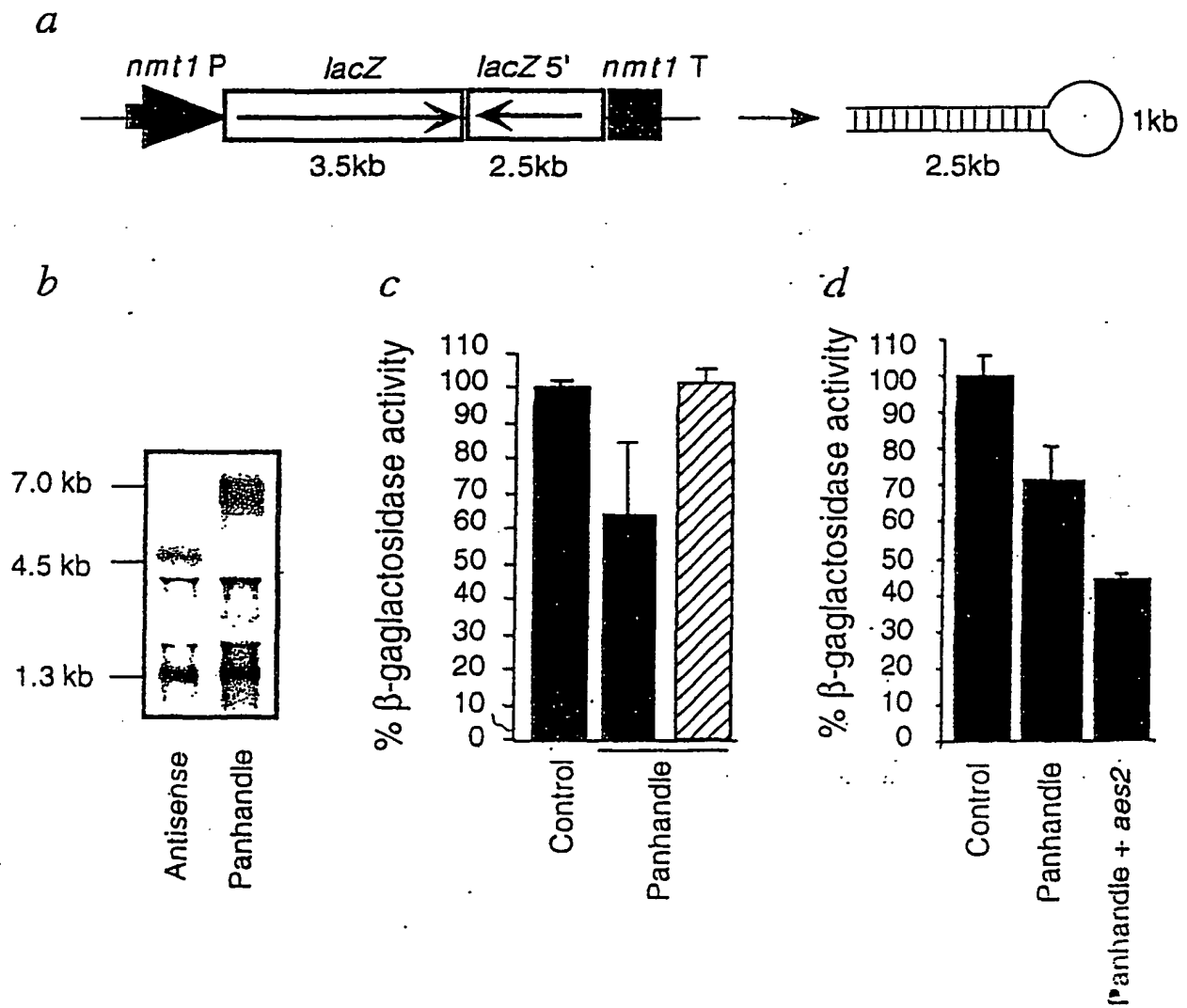


Figure 10

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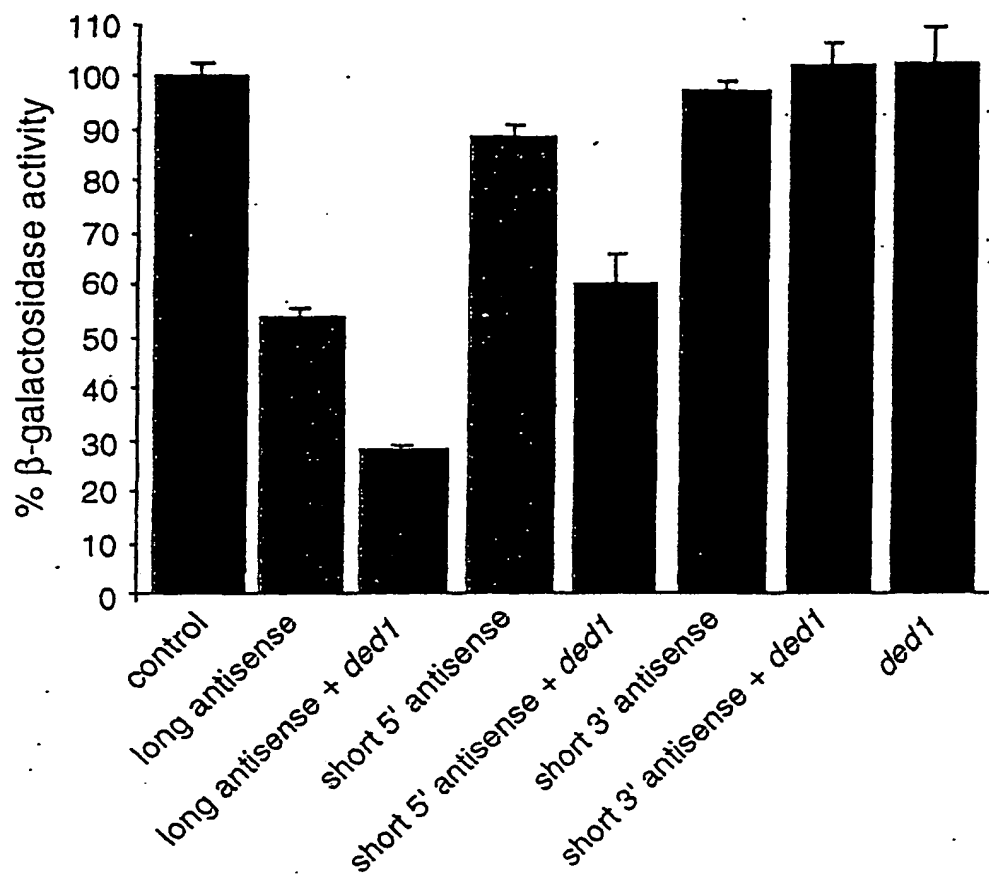


Figure 11

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DNA sequence for aes1 factor (Seq ID No. 1)

TTTAACTTAGTTCGCTTTGTTAAATTGGCCTCGAGGTCGACGTTAATTAAGCCGCAATT
GTAACAGTAGATTTTTTGCATCATTATTACTCTCCGAAACATGACTGAACACTCATTTA
5 AGCAAATAGACGTGTTTTCTAATAAAGGTTTTTCGAGGTAATCCTGTTGCAGTTTTTTTT
GATGCAGATAATTTATCACAAAAGGAAATGCAGCAGATTGCCAAGTGGACAAATTTATC
TGAGACAACATTTGTTCAAAGCCGACAATCGATAAAGCAGATTACAGACTTCGTATAT
TTACCCCAAGAATGTGAATTAAGCTTTGCTGGTCACCCAACAATTGGATCGTGCTPTGCT
GTTGTTGAAAGTGGATATTGTACTCCAAAAAAGTGTAAATTATTTCAGGAATGTTTAGC
10 CGGTTTAGTTGAATTAAGTATCGATGGGGAAAAGGATGAAGACACTTGGATTTCTTTCA
AACTTCCGTATTACAAAATTTTACAGACTTCTGAACTGCAATTTCAGAAGTAGAAAAT
GCATTGGGTATTCCTCTGAATTATAGTTCTCAAGTTTCTCCTCCTGTGTTAATAGATGA
TGGACCAAAGTGGCTTGTAATTCAACTTCCAAACGCTACAGATGTGCTCAACCTCGTTC
CGAAATTTAGTCCCTTTCCCAAGTTTGTAAAAACAATGATTGGATAGGCGTCACCCGT
15 CTTTGGTGAATTAGAAAAGACTCGTTTGAAAGCCCGAAGCTTTGCGCCTTTAATACATG
TCAATGAGGATCCGGCTTGCGGTAGTGGTGCAGGAGCTGTCGGTGTGTATATTGGAAGC
TCTCAAAAAACTCCAACCTCTCTATCATTTACGATTTCTCAAGGTACAAAATTAAGTAG
ACAAGCAATTTCCAAAGTCAGCGTAGACGTTTCTCCTCCAATAAATCAATTGCTGTTTTTG
TCGGTGGACAGGCAAAAACTTGTAATTTCTGGAAAATCGTTTATTTAATGTTTTTATTAC
20 AAATATTTCACTTGCAGATTTATTTTCCAATACTGAAGACTTTCAATCAATAGCAAATAT
GCTACTCAAGGAAGTTCACTCATTCAAAGCAATTGGTTTACTATATCGTTTTTTCTAA
CTAGTTACTAGTCATTGAACAATCTACCGAATGATAAAATGAAATTTTGGTTTTTCCCC
GGGTAAAAGGAATGTCTCCCTTGCCAGTACTGCTAGGGTTTTTCTTTTCTGAAGTATAAGA

DNA sequence for aes2 factor (Seq ID No 2)

5 AGTCCGCTTTGTTAAATTGGCCTCGAGGTCGACGTTAATTAAGCCTGATATGATCGAGC
TTGTCGAAATGGAAATGCGTGAGCTACTCTCCGAATACGGATTTGATGGTGACAATACT
CCAATTGTTAGCGGCAGTGCTTTATGTGCCTTAGAGGGTCGTGAGCCTGAGATTGGTCT
CAATAGTATTACTAAATTGATGGAAGCTGTTGATAGTTATATTACTCTTCCTGAAAGAA
AAACGGATGTCCCTTTCTTGATGGCCATCGAGGACGTTTTTTCAATTTTCAGGTCGCGGA
ACTGTAGTCACTGGCCGTGTCGAGCGCGGTACTTTAAAGAAGGGTGCTGAAATCGAAAT
10 CGTCGGTTATGGTAGCCATTTAAAGACTACCGTTACTGGAATTGAAATGTTCAAAAAGC
AGCTTGATGCCGCCGTTGCCGGTGACAATTGTGGCCTTTTACTTCGTTCTATCAAGCGA
GAGCAATTAAAACGTGGAATGATTGTGCTCAACCAGGAACCGTTGCTCCTCATCAGAA
ATTCAAGGCATCATTTCTATATTTTGACAAAAGAGGAAGGAGGTCGTCGTACCCGGTTTC
GTTGACAAGTATCGTCCCCAACTGTACAGTCCGTACTTCCGACGTTACTGTGCAACTTA
15 CCCACCCTGATCCTAACGACTCAACAAAATGGTTATGCCTGGAGACAATGTCGAGATGA
TCTGTACGCTTATTCACCCCATTGTCATCGAAAAAGGACAACGCTTCACAGTTCGTGAG
GGTGGAAGCACTGTAGGCACAGCTTTGGTTACTGAACTTTTGGATTAGTGCATTTATGA
ACTTATTGGCTTTAAAAATTTTGCATGCTGAATACCAATATTATGTCCTTCTCAGAAT
TCTATAACTACAGTGTCAATTATTGTAATAAGACTTTTGCATCCATTGACAATGGTATTT
20 GATACTTTTATAGTTTCTACTATTGTTAGCCAAAGTTATAAAACAAATAATAAAATAAC
GTTGAATCAAAAAAAAAAAAAAAAAAAGCGGCCGCGGATCCCCGGGTAAAAGGAATGTC
TCCCTTGCCAGTACTGCTAGGGTTTTTCTTTCAAACATATGGGA

DNA sequence for aes3 factor (Seq ID No 3)

ATTTTCAGACGCAATTCACATGGCTTTTGGACTGTATTGCTATTCTTGTCTGGTTTAGTTGC
5 TACGACGCTTGCCAAGATGCCTCTAAATTATGCTTACCCCTTTGGATTTGCAAAAATTG
AGGCTCTTTTCGGGTTTCACTAATGGTATTTTTTTTAGTTTTGATTTTCATTTTCTATCGTC
GGCGAGGCATTATATAGGTTATTTTCATCCGCCCCAAATGAATACCGACCAATTGTTGTT
GGTTAGTTTTTTTGGGCCTTGTTGTGAATTTGGTAGGTATCCTAGCGTTCAATCATGGGC
ATAATCATGATCATGGGTCTCATCACCATCATTCCCATAGTAATCATAGTATGTGTCTG
10 CCTAACACTACAAATGATATAAATATTTTTGAAGAGTTTGAAGAAGAAAAAGATAATGT
TGAAGCCCAGAAAATGGGCTATACGAATGACGATCACGTATCCCAACATGAACATACCC
ATGAGAATAGTCAGGAACATCACCATGAGCATAACCACAATCATGATCACATCCATAAA
TACAATGAAAAATGCGACCATGAAAGCATAAGTCTCCAGAATTTAGACAATGATCATCA
CTGTCATCATCACCATGAAAATCATAATATGCATGGCATATTTCTGCATATTATCGCAG
15 ATACTATGGGCTCTGTTGGAGTTATTGTCTCTACTATATTAATACAGTGGTTTTTCATGG
ACCGGTTTTGATCCTTCGGCATCTCTAATAATTGCTGCATTAATATTTGTTTTCTGTACT
TCCATTAATTAAAGATTCTGGCGAAGAATTTGCTCTCTGTGACTGATCCAGAATCGGAAT
ATTTATTGAAGCAGTGTTTGTCGAACATCAGTTTAAGTCACTCCGTTGTCAGTTTATCC
AACCCTAAGTTCTGGACAAACGAAAGAGGTGAAGTGTATGGAATACTCCATATTCAGGT
20 GAGCATAGACGGTGATTTAAACGTGGTTCGTAATGAAGTATTTAGGAAGCTCTCAATCG
CTGTACCAAATTTAAACACATTTGTATACAATCTGAACGGCCAAACAATTGCTGGTGT
GGAAAATAGTTCTTACATCAGTTGATATCCATACTTATTTACGTGTAATTTTAATTAGA
TGAATTAATATTTTCTTTATTAGC

DNA sequence for aes4 factor (Seq ID No.4)

TTTACTTTAGTCGCTTTGTTAAATTGGCCTCGAGGTCGACGTTAATTAAGCTTTTTTTT
5 TAAGAGATATAACATATGTCAACGCGTCATTGATTAAC TACATAACACGCCAATTATAA
ACTTCTCCCAAAGA ACTTAAGAATTTCATTTTCAATCCAGATGAATTTATTTAAGAG
ACGAACAGTAGCGGCAGCAGCCTTAGCACGCTTAGCGAGCAAAGCTTGGGTCTTACCAC
CCATGATACCCACCACCCCACTTACGACGGGCTTTCGTCTGTA CTTAGCAGAGAAGTTAG
CATCAACGGCGGAGACAATAGAAGCGAGTTCGTTCTTGTCTTCCTCACGGACCTCAGTG
10 ACAGCTAAACAGCAGCAGTCTTTTGGTGAATGACAGTACCAAGGCGGGCCTTGTCTT
GACAATGGCATAAGGAACACCCATCTTCTTGACAAAGCAGGCAAGAAAACGACGAGTT
CAATGGGGTCGACATCGCTGGCAATGAGAACCAACTTAGCCTTCTTGGCCTCAATGAGA
GCTACAACATGGTTCAAACCATATTTAACATTGTAAGGCTTCTTAGAGACGTCTTGAGC
AGACTTGCCGTTGGCAACAGCCTCGGCTTCAGCAACCAAACGTTGCTTCTTTTCAGCAG
15 CAGTCTCAGGACGGTACTTGTTAAGCAACTTGAAGACCTGAGTAGCAGTGTTTTGTCC
AAAGTCTTCTGGAACTGAGCAATGGCAGGAGGAACCTTCAAACGCAAGTTCAAAATCTT
GCGACGGCGTTGAAGGCGGATATACTCAGGCCACTTAACAAAACGGCTCAAGTCACGCT
TAGGTTGGATGTCTTGTCCCCCGGGTAAAGGAATGTCTCCCTTGCCAGTACTGCTAGG
GTTTTTCGTTCGAATAAGGCC

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